Transcranial magnetic stimulation of the precuneus enhances memory and neural activity in prodromal Alzheimer's disease

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Running title: Precuneus modulation in AD

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Abstract

Memory loss is one of the first symptoms of typical Alzheimer's disease (AD), for which there are no effective therapies available. The precuneus (PC) has been recently emphasized as a key area for the memory impairment observed in early AD, likely due to disconnection mechanisms within large-scale networks. Using a multimodal approach we investigated in a two-week, randomized, sham-controlled, double-blinded trial the effects of high-frequency repetitive transcranial magnetic stimulation (rTMS) of the PC on cognition, as measured by the Alzheimer Disease Cooperative Study Preclinical Alzheimer Cognitive Composite in 14 patients with early AD (7 females). TMS combined with electroencephalography (TMS-EEG) was used to detect changes in brain connectivity. We found that rTMS of the PC induced a selective improvement in episodic memory, but not in other cognitive domains. Analysis of TMS-EEG signal revealed an increase of neural activity in patients' PC, an enhancement of brain oscillations in the beta band and a modification of functional connections between the PC and medial frontal areas.

Our findings show that high-frequency rTMS of the PC is a promising, non-invasive treatment for memory dysfunction in patients at early stages of AD. This clinical improvement is accompanied by modulation of brain connectivity, consistently with the pathophysiological model of brain disconnection in AD.

Keywords: AD, memory, precuneus, transcranial magnetic stimulation

Abbreviations: Alzheimer's disease (AD), default mode network (DMN), precuneus (PC), repetitive transcranial magnetic stimulation (rTMS), magnetic resonance imaging (MRI), cerebrospinal fluid (CSF), mini mental state examination (MMSE), first dorsal intereosseous (FDI), resting motor threshold (RMT), standard deviation (SD), electroencephalography (EEG), Rey Auditory Verbal Learning test (RAVLT), Alzheimer Disease Cooperative Study Preclinical Alzheimer Cognitive Composite (ADCS-PACC), Digit Symbol Substitution Test (DSST), Frontal

Assessment Battery (FAB), theta/alpha transition frequency (TF), individual alpha frequency (IAF), TMS-evoked potentials (TEPs), inter-stimulus interval (ISI), posterior parietal cortex (PPC), global mean field power (GMFP), event-related spectral perturbation (ERSP), inter-trial coherence (ITC), standardized low resolution brain electromagnetic tomography (sLORETA), Montreal Neurological Institute (MNI), long-term potentiation (LTP).

1. Introduction

Alzheimer's disease (AD) typically presents with deficits in learning new information as well as in retrieving old memories (Bäckman et al. 2001). This loss in long-term episodic memory has been referred not only to local damage of the medial temporal lobes, but also to a dysfunction of large-scale networks underlying memory processes. Since the early stages of AD, prominent neuropathological abnormalities (i.e., β-amyloid plaques and neurofibrillary tangles) are known to affect the posterior cortical regions of the brain, including the precuneus (PC), the posterior cingulate, the retrosplenial, and lateral posterior parietal cortex (PPC) (Buckner et al. 2005). These abnormalities are paralleled by an initial disruption of medial fronto-parietal functional connectivity, as revealed by alterations of the so-called default mode network (DMN), for which the PC is a key node (Buckner et al. 2008; Raichle et al. 2001). The disconnection of the PC precedes (and probably contributes to) the occurrence of regional brain atrophy, which becomes prominent at later disease stages (Gili et al. 2011). Indeed, AD patients often show a reduction of PC cortical thickness accompanied by an abnormal activity during memory task performance, and a decrease in functional connectivity (Chen et al. 2017). This is relevant since the activity of the PC is considered necessary for episodic memory retrieval (Lundstrom et al. 2005; Wagner et al. 2005). Therefore, the PC is a vulnerable region for the transitional stage towards dementia, and might represent an ideal target for tailored interventions aimed at counteracting AD-related memory decline.

So far, the only approved treatment for AD is based on cholinergic and glutamatergic drugs. Yet, these drugs have negligible efficacy on memory deficits, and alternative strategies are needed to improve memory in patients with AD (Canter et al. 2016). Recently, non-invasive brain stimulation methods have been proposed as a novel approach to improve some cognitive performances in patients with dementia (Cotelli et al., 2006; 2008; Ferrucci et al., 2008; Turriziani et al., 2012) and in healthy volunteers (Casula et al., 2017a; Rastogi et al., 2017). These studies provided evidence that repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex (DLPFC) may transiently improve language functions, such as naming and

sentence comprehension (Cotelli et al., 2011). More recently, other studies applied rTMS over different brain areas involved in the cognitive decline of AD patients (i.e. right and left DLPFC, right and left posterior parietal cortex (PPC) associative areas, and Broca and Wernicke language areas) in combination with adapted cognitive training (Bentwich et al., 2011). These studies showed promising results in terms of global cognitive functions as indexed by the improvement of the ADAS-Cog score after 5 weeks of treatment (Nguyen et al., 2017; Rabey et al., 2013). However, this approach did not show any specific effect on memory functions.

Here we tested the hypothesis that rTMS of the precuneus (PC) may be a novel target to treat memory dysfunction in AD patients. This finds support in recent evidence showing that rTMS applied to key nodes of the DMN such as the PPC (Wang et al. 2014; Nilakantan et al. 2017) and PC (Rose et al. 2016; Bonnì et al. 2015) improves short and long-term memory functions in healthy individuals. Moreover, we recently demonstrated that rTMS of the PC exerts its effects not only at local but also at a network level by modulating the activity of the PC and its connections to other brain areas (Mancini et al., 2017). Therefore, we hypothesized that high-frequency excitatory rTMS of the PC might improve long-term memory in patients with AD, by modulating the neural activity of the PC and its connections with medial parietal and frontal areas. To evaluate the neurophysiological modifications induced by rTMS and potentially underpinning behavioral changes, we used single-pulse TMS combined with EEG recordings.

2. Materials and Methods

2.1 Experimental Design and participants

The current study was approved by the Ethical Committee of Santa Lucia Foundation (Protocol number: CE/PROG.465). Written informed consent was obtained from all patients before entering the study. Neuronavigated rTMS was used to stimulate the PC of AD patients for two weeks in a sham-controlled crossover design. Thirty patients, admitted to the Specialist Memory Clinics of "Tor Vergata" University (Rome, Italy) and Catholic University of Rome (Rome, Italy) between January 2014 and June 2016 for complaining of memory symptoms, were screened for the current study. Patients' recruitment was performed according to current diagnostic criteria for prodromal AD (Dubois et al. 2016), referring to the early symptomatic phase of AD, characterized by episodic memory loss in the presence of AD pathology as supported by CSF or imaging biomarker evidence. All AD patients had to be cognitively intact before the occurrence of cognitive impairment. Subjective memory impairment, corroborated by an informed caregiver had to occur at least 12 months earlier. After the first visit, patients underwent, for diagnostic purposes, a complete clinical investigation, including medical history and neurological examination, a complete blood screening, a neuropsychiatric evaluation, brain MRI scanning, and an extensive neuropsychological assessment exploring all cognitive domains (Table 1). Lumbar puncture was performed in all patients to confirm the typical CSF profile of AD pathology, i.e., reduced concentrations of amyloid $\beta_{1\text{-}42}$, increased levels of total- and phosphorylated-tau. Major systemic and psychiatric disorders, other neurological conditions, and signs of concomitant cerebrovascular disease on MRI scans were carefully investigated and excluded in all patients. Patients who agreed to participate (N=14; see Table 2) were randomly assigned to rTMS or sham as their first experimental arm belonging. The order of administration of either intervention (i.e., rTMS or sham) was counterbalanced across all patients. rTMS (or sham) was applied daily, at the same day-time, in a 10 session course, Monday to Friday. A two-week washout interval was applied before patients were crossed over to either experimental arm for two more weeks. Neuropsychological and neurophysiological evaluations, the

latter performed by combining TMS and EEG, were performed on the Monday morning before and after each of the two week-treatment (rTMS or sham). All researchers performing patients' evaluations were blind to their experimental arm belonging. Details of the study design are summarized in Figure 1A.

2.2 Intervention - Repetitive TMS

rTMS was carried out using a Magstim Rapid^2 magnetic biphasic stimulator connected with a figure-of-eight coil with a 70-mm diameter (Magstim Company, Whitland, UK) that generates 2.2 T as maximum output. Each daily stimulation session consisted of 42-sec trains delivered at 20 Hz spaced-out by 28 seconds of no stimulation (total number of stimuli: 1600). The entire session lasted approximately 20 minutes. Intensity of stimulation was set at 100% of the resting motor threshold (RMT), defined as the lowest intensity producing MEPs of >50 μV in at least five out of 10 trials in the relaxed first dorsal intereosseous (FDI) muscle of the right hand (Rossini et al. 2015). RMT was assessed over the optimal stimulus site to elicit MEPs in the right FDI, termed "motor hotspot", identified by positioning the coil approximately over the central sulcus and moving it on the scalp by 0.5 cm steps on left M1.

During the rTMS treatment, the coil was positioned over the PC and constantly monitored using the Softaxic neuronavigation system (EMS, Bologna, Italy) coupled with a Polaris Vicra infrared camera (NDI, Waterloo, Canada). Individual T1-weighted MRI volumes were used as anatomical reference (Figure 1B). Since the coil-to-cortex distance directly affects the magnitude of magnetic stimulation, for each patient we calculated a distance-adjusted MT (AdjMT). $AdjMT=MT+m\times(DsiteX-DM1),$ where *AdjMT* is the adjusted MT in % of stimulator output, *MT* is the unadjusted MT in % of stimulator output, *DM1* is the distance between the scalp and M1 hotspot, *DSiteX* is the distance between the scalp and a second cortical region (SiteX), and *m* is the distance-effect gradient. This procedure provides a more accurate index of cortical excitability and improves the efficacy of MT-calibrated TMS (Stokes et al. 2005). Coil orientation was parallel to

the midline with the handle pointing downward. For sham treatment, stimulation was applied using the same parameters with the sham coil positioned to the scalp in correspondence to the target area. For each patient, a source estimation on pre-processed TMS-EEG data was run at the beginning of each treatment session to confirm the correct anatomical targeting for rTMS.

2.3 Cognitive Evaluations

To evaluate the behavioural effects of the rTMS, we employed a battery of tests assessing long-term episodic memory, executive functions, attention, and global cognition, according to the Alzheimer Disease Cooperative Study Preclinical Alzheimer Cognitive Composite (ADCS-PACC) (Donohue et al. 2014). This battery is a well-known clinical instrument with high sensitivity to cognitive decline in prodromal and mild dementia, and with a sufficient range to detect early decline in preclinical disease stages. The battery includes: 1) the immediate and delayed total recall score from the Rey Auditory Verbal Learning test (RAVLT), to evaluate long-term episodic memory; 2) the Digit Symbol Substitution Test (DSST) from the Wechsler Adult Intelligence Scale–Revised, to evaluate response speed, sustained attention, visual-spatial skills, and set-shifting; 3) the Mini Mental State Examination (MMSE), to evaluate global cognition, and 4) the Frontal Assessment Battery (FAB), to evaluate executive functions.

2.4 EEG recordings

EEG was performed using a TMS-compatible EEG equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany), and was continuously recorded from 29 scalp sites positioned according to the 10-20 International System. TMS-compatible Ag/AgCl pellet electrodes were mounted on an elastic cap, while additional electrodes were used as ground and reference. Horizontal and vertical eye movements were detected by recording the electrooculogram (EOG) to off-line reject trials showing ocular artifacts. The ground electrode was positioned in AFz, while the reference one was positioned on the nose tip. The EEG and EOG signals were band-pass filtered at

0.1-1000 Hz and digitized at a sampling rate of 5 kHz. Skin/electrode impedance was maintained below 5 kΩ.

Resting EEG

Resting EEG was recorded before each TMS/EEG session for 3 minutes with open eyes. As a first step, data were downsampled to 1000 Hz and band-pass filtered between 1 and 80 Hz (Butterworth zero phase filters). A 50 Hz notch filter was also applied to reduce noise from electrical sources. Identification and removal of artifacts (muscle activity, eye movements and blink-related activity) was made with independent component analysis (INFOMAX-ICA). Then, signal was segmented in 2-sec epochs. Power density was estimated by means of the Fast Fourier Transform (10% Hanning-window) from 4 to 45 Hz, with a frequency resolution of 0.5 Hz. For each participant, frequency bands were established based on two subjective anchor frequencies, which is the theta/alpha transition frequency (TF) and the individual alpha frequency (IAF) peak (Klimesch, 1997). The TF represents the minimum power in the alpha frequency range, whereas the IAF represents the frequency with the maximum power peak within the alpha range (7-12 Hz). Based on TF and IAF, we estimated the frequency band range for each participant, as follows: delta, from TF-4 to TF-2; theta, from TF-2 to TF; low alpha, from TF to IAF; high alpha, from IAF to IAF+2. For the estimation of individual beta and gamma frequencies we detected three peaks in the frequency range from IAF+2 to 45 Hz, these peaks were named beta1, beta2 and gamma peak. Low beta range was computed from IAF+2 to the lower spectral power between beta1 and beta2 peak; high beta range was computed from beta 1 to the lower spectra value power between beta 2 and gamma peak; gamma range was computed from beta 2 to 45 Hz. The mean band power was then obtained by averaging the power values of all the single-trial epochs for each participant.

TMS/EEG

To evaluate the neurophysiological modifications induced by rTMS and potentially underpinning behavioral changes, we used single-pulse TMS combined with EEG recordings. During all neurophysiological evaluations, patients were seated on a dedicated, comfortable armchair in a soundproof room. Each neurophysiological assessment began with a TMS-EEG session to evaluate the cortical excitability and the oscillatory activity evoked by single-pulse TMS. Single-pulse TMS was carried out using a Magstim Rapid^2 magnetic biphasic stimulator connected with a figure-of-eight coil with a 50-mm diameter (Magstim Company, Whitland, UK). Each TMS-EEG session consisted of 80 single pulses applied at a random inter-stimulus interval (ISI) of 2-4sec. TMS-EEG was applied over the PC, which was the target of the rTMS intervention, and over the left posterior parietal cortex (l-PPC). Importantly, we chose to stimulate the l-PPC as a control site for its proximity to the PC, since we want to assess the spatial specificity of our rTMS intervention. The order of stimulation of either area was counterbalanced across patients. The intensity of stimulation of single-pulse TMS was set at 90% of the AdjMT (PC: 57.8±3.1; l-PPC: 54.2±2.6). A short break was run between TMS-EEG stimulation of either site. The coil was differently oriented respect to the mid-sagittal axis of the patient's head, for each stimulation site: parallel over the PC, at 15° over l-PPC, with the handle pointing backward. The coil position was constantly monitored using the Softaxic neuronavigation system, to ensure a high degree of reproducibility across neurophysiological assessments.

TMS-EEG data were preprocessed offline using Brain Vision Analyzer (Brain Products GmbH, Munich, Germany). TMS-EEG data were analyzed offline with Brain Vision Analyzer (Brain Products GmbH, Munich, Germany) and EEGLAB toolbox running in a MATLAB environment (MathWorks Inc., Natick, USA; Delorme and Makeig, 2004). As a first step, a cubic interpolation from 1 ms before to 10 ms after the TMS pulse was applied to remove the TMS artifact. Afterwards, the signal was downsampled and filtered as on the resting EEG data. Physiological and TMS-related artefactual components were detected using INFOMAX-ICA and removed basing on their scalp distribution, frequency, timing and amplitude (Casula et al., 2017b).

Data were then segmented into epochs starting 1 s before the TMS pulse and ending 1 s after it. Afterwards, all the epochs were visually inspected and those with excessively noisy EEG were excluded from the analysis, resulting 68.7±2.9 in the pre-rTMS condition, 68.9±2.9 in the post-rTMS, 70.7±2 in the pre-sham, 70.1±2.1 in the post-sham, stimulating the PC. For l-PPC stimulation, we used 76.5±1.1 trials in the pre-real condition, 76.1±1.9 in the post-real, 76.4±0.8 in the pre-sham, 76.9±1.4 in the post-sham.

Two sets of outcome measures were obtained, assessing, respectively, cortical excitability (using a spatio/temporal-domain analysis) and cortical oscillatory activity (using a time/frequency-domain analysis). To evaluate changes in cortical excitability, we evaluated both, global and local cortical responses evoked by TMS. The global response was assessed, for each patient and each stimulation site (PC and l-PPC), by measuring the first five peaks (10-35 ms for P1; 36-62 ms for P2; 63-90 ms for P3; 91-165 ms for P4 and 166-250 ms for P5) of the global mean field power (GMFP) waveform within the 300 msec following the TMS pulse. Local responses were measured by the TMS-evoked potentials (TEPs) waveform at each electrode within the five temporal windows used for GMFP analysis.

To evaluate changes in the oscillatory domain, we performed a time/frequency decomposition based on Morlet wavelet (parameters c=3; 41 linear 1 Hz steps from 4 to 45 Hz), and then we computed event-related spectral perturbation (ERSP) and inter-trial coherence (ITC). ERSP is a measure of event-related changes in spectral power over time in a certain frequency range computed as:

$$TRSP(f,t) = \frac{1}{n}\sum_{k=1}^{n}|F_k(f,t)|^2$$

Where, for n trials, the spectral estimate F was computed at trial k, at frequency f and time t. ITC is a measure of the partial or exact synchronization of activity at a particular latency and frequency to an experimental event to which EEG data trials are time locked (i.e. TMS pulse), computed as:

$$ITC(f,t) = \frac{1}{n}\sum_{k=1}^{n}\frac{F_k(f,t)}{|F_k(f,t)|}$$

ITC coefficient takes value between 0 and 1, where 0 represents absence of synchronization between EEG data and the time-locked event and 1 represents their perfect synchronization. Analysis of ERSP values was conducted over five regions of interest (ROI), each one comprising three electrodes: frontal ROI (F3, Fz, F4); central ROI (C3, Cz, C4); parietal ROI (P3, Pz, P4); left ROI (F7, T7, P7) and right ROI (F8, T8, P8). To minimize the effect of possible artifacts occurring at the time of stimulation, the frequency values were averaged over a 20-250 ms time window from the TMS pulse, corresponding to the timing of the oscillatory activity. The spectral power was computed in the frequency ranges between 4-7 Hz (theta), 8-13 Hz (alpha), 14-30 Hz (beta) and 31-45 Hz (gamma).

2.5 Statistical Analysis

To assess the effect of rTMS on patients' performance at RAVLT, repeated-measures ANOVAs with treatment (rTMS or sham) and time (pre- or post-treatment) as factors were performed. The same test was used to assess changes in the patients' performance at all tests included in the ADCS-PACC, to ensure that the improvement induced by rTMS was specific for episodic memory.

To assess the rTMS effect on the spectral power of resting EEG we used a repeated-measures ANOVAs with factors treatment (rTMS and sham) and time (pre- and post-treatment) separately conducted for each frequency band (delta, theta, low alfa, high alfa, low beta, high beta and gamma). Effects on amplitude of GMFP peaks (global response) were measured with a repeated-measures ANOVA with factors treatment (rTMS and sham), time (pre- and post-treatment) and peak (P1, P2, P3, P4, P5) performed separately for the two stimulation sites (PC or l-PPC).

To assess the treatment effects on local cortical response over the PC and the l-PPC, we used multiple dependent t-tests comparing TEPs waveform at each electrode within the five temporal

windows used for GMFP analysis. Non-parametric, cluster-based permutation statistics was conducted to correct for multiple comparisons (Maris et al. 2007). This method performs a non-parametric statistical test by calculating Monte Carlo estimate of the significance probabilities from two surrogate distributions constructed by randomly permuting the two original conditions data for 3000 times (Casula et al., 2017c). The clusters for permutation analysis were defined as the two (or more) neighboring electrodes in which the t-value at a given time point exceeded a threshold of $P<0.05$.

Repeated-measures ANOVA with factors region-of-interest (frontal, central, parietal, left, right), treatment (rTMS or sham) and time (pre- or post-treatment) were performed to evaluate the rTMS effects on oscillatory activity (ERSP), separately for each frequency band. Analysis of ITC was conducted on the beta-band activity (14-30 Hz) considering the electrode over the stimulation site, i.e., Pz for the PC and P3 for l-PPC. Repeated-measures ANOVA with treatment (rTMS or sham) and time (pre- or post-treatment) as factors was performed to evaluate the effects of rTMS on ITC beta-band activity.

For all the ANOVA procedures, the Huynh–Feldt ε correction factor was applied where appropriate to compensate for effects of non-sphericity. Bonferroni's correction was applied to post-hoc comparisons.

2.6 Source analysis

In order to confirm the targeting of the stimulation, as a preliminary analysis, we used source estimation on the pre-processed TMS-EEG data at the beginning of the treatment for each subject. Briefly, using BrainStorm software we estimated the activity of the dipoles underlying the brain electrical activity, by means of the ICBM152 template (Tadel et al., 2011). Since this kind of inverse problem presents an infinite number of dipole patterns that could generate the same electrical activity registered by the electrodes, we used minimum norm imaging to approach the forward modelling. As a preliminary step, this method finds a cortical current source density

distribution that can approximate the data, and then defines the solution with the minimum energy, using source covariance. We modelled one dipole at each grid point, normally oriented to the cortical surface. We then used the standardized low-resolution brain electromagnetic tomography (sLORETA) measure to normalize the current density map at each point (Pascual-Marqui et al., 2002). The estimated source distribution was averaged across the patients (Supplementary Movie 1) and the PC was selected as region of interest using the definition of the Desikan-Killiany atlas (Desikan et al., 2006).

2.7 Behavioral-neurophysiological correlations

In order to investigate possible relationships between clinical and neurophysiological outcomes, we tested correlations using the Pearson's coefficient (two-tailed) between the modulations observed in the RAVLT and in the GMFP.

3. Results

Fourteen patients with AD (F/M=7/7) took part in the study, which was conducted between January 2014 and June 2016. Demographic and clinical characteristics of the cohort are summarized in Table 1. They all completed the protocol successfully, attending all experimental sessions.

3.1 Source analysis

The source analysis of TMS-evoked EEG activity showed that the response was spatially distributed along the wall of the medial superior parietal cortex bilaterally, corresponding to the PC (Movie 1) (Bzdok et al. 2015). This confirmed, in all participants, a correct anatomical identification of the stimulation site (Figure 1C). The Montreal Neurological Institute (MNI) coordinates of the stimulation site averaged across participants were [x,y,z]=[0,-65±2,37±9]. The mean scalp-to-cortex distance for the PC was 27±0.3 mm, and the AdjMT for rTMS was 60.8±3.1% of maximum stimulator output.

3.2 Cognitive evaluation

ANOVA on the performance at Delayed Recall of the Rey Auditory Verbal learning Test showed a significant time × treatment interaction [F(1.13)=5.98; p=0.029] (Figure 2). Post-hoc analysis revealed a significant improvement at the test performance after rTMS (pre vs. post, 2.42±0.8 vs. 3.14±0.8). No significant effects were detected after sham stimulation (post vs. pre, 2.86±0.7 vs.2.50±0.8). No significant effects were observed on patients' executive functions, attention or global cognition (all ps>0.05, table 3).

Comment [Cla1]: Credo sia SEM

Comment [Cla2]: SEM

Comment [Cla3]: Questo credo sia SEM (standard error mean) e non SD perché in Table 3 Sd è 2.7 o 2.9

Comment [Cla4]: SEM

3.3 Resting EEG

ANOVAs performed on the mean spectral power for each frequency band, did not reveal any significant main effect of rTMS (all ps>0.05), time (all ps>0.05), nor any significant interaction between the two factors (all ps>0.05).

3.4 TMS-evoked cortical activity

ANOVA performed on mean TMS-evoked activity evaluated over PC revealed a significant rTMS × time × peak interaction [F(4.52)=5.74; p =0.0006], due to a significant increase of P3 after real rTMS treatment (Figure 3a). Post-hoc analysis showed for P3 a significant difference between pre and post real rTMS treatment (p=0.008), and between post real and post sham (p=0.005). For all peaks, no significant differences were observed between baseline of each rTMS treatment (all ps>0.05) and between pre and post sham rTMS (p>0.05, Ffigure 3b). No significant effects were found when stimulating l-PPC indicating that the rTMS protocols was unable to induce a general effects on cortical excitability of surrounding cortical sites (Ffigure 3c,d). When stimulating the PC, the cluster-based analysis did not reveal any significant difference between the baselines of the two groups (i.e. pre-sham rTMS vs. pre-real TMS; Monte Carlo p>0.05). As revealed by TEPs and GMFP amplitude, PC-rTMS produced a significant increase of TMS-evoked activity from PC over a specific time window, i.e. from 60 to 90 ms after TMS over two distinct cluster of electrodes: one frontal, comprising Fz, FC2 and F4 electrodes; and one parieto-occipital, comprising Pz, O1 and O2 (Monte-Carlo p<0.01). No difference was detected after sham stimulation, nor after stimulation of l-PPC (Monte-Carlo p>0.05).

3.5 TMS-evoked oscillatory activity

ANOVA performed on mean ERSP values evaluated over PC revealed a significant ROI × rTMS × time interaction when beta oscillatory activity was evaluated [F(4.52)=3.093; p=0.023]. Post-hoc analysis showed a significant increase of beta activity over the parietal ROI in the post- rTMS condition compared to pre- (3.565±0.37 vs. 2.257±0.46 μV; p=0.021) (Figure 4a). No effect was revealed in the sham rTMS condition (Figure 4b), nor between the baselines of the two conditions (pre-real rTMS vs. pre-sham rTMS; p>0.05). ANOVA did not reveal any significant differences in the other frequency bands (all ps>0.05).

ANOVA performed on mean ITC values evaluated over PC revealed a significant rTMS × time interaction when beta oscillatory activity was evaluated [$F(1.13)=11.36$; $p=0.005$]. Post-hoc analysis showed a significant increase of beta activity after rTMS condition compared to pre- (0.0006±0.01 vs. 0.007±0.01; $p=0.002$) (Figure 4c). Again, no significant effects were observed after sham treatment (Figure 4d) nor when stimulating the l-PPC (Figure 4e-h).

3.6 Behavioral-neurophysiological correlations

Analysis of relationships between clinical and neurophysiological data did not reveal any significant correlation (all $ps>0.05$).

4. Discussion

We evaluated here the usefulness of rTMS in modifying selectively the cognitive performance of patients with typical AD at early clinical stages. In our patient sample, we demonstrated a significant beneficial effect of this intervention in improving episodic memory. Our neurophysiological data suggest that this improvement is underpinned by changes in cortical activity of the PC and its connectivity with frontal areas.

rTMS induced an average increase of 0.8 recalled items (36%) at RAVLT (delayed recall). These results provide the first evidence that rTMS could be used as a non-pharmacological intervention to counteract memory loss in AD. The study design was rigorous, with rTMS being sham-controlled in a cross-over design. AD patients, as well as experimenters performing evaluations were blind to the treatment condition at any time of the study – thus minimizing the risk of observing a placebo effect. Despite the sample was relatively small, these results are robust, and could provide the basis for planning a clinical trial with a between-group design aimed at evaluating the potential beneficial effects of rTMS of the PC in slowing cognitive decline when applied during a longer period (i.e. six months).

In all AD patients, rTMS delivery on the PC was strictly verified in terms of anatomical localization by a source reconstruction analysis of TMS-evoked EEG activity, using a neuronavigation system. Critically, cognitive evaluations were constantly paralleled by TMS-EEG monitoring, thus allowing a concomitant assessment of the clinical effect of rTMS alongside with information on neurophysiological modulation of the brain. Taken altogether, these findings provide novel evidence that non-invasive treatment of network dysfunction, through stimulation of the PC, represents an effective strategy to enhance long-term memory in AD.

The rTMS-induced improvement of long-term memory reinforces the notion that PC is directly involved in memory dysfunction in prodromal AD (Lundstrom et al. 2005). With this regard, recent models of long-term memory showed that, in healthy conditions, the encoding of episodic memory is associated with reduced PC activity, while the retrieval is associated with

increased PC activity (Daselaar et al. 2009). This interaction, which has been termed “the encoding/retrieval flip” (Huijibers et al. 2012), is reduced in elderly adults with amyloid pathology (Vannini et al. 2012). Moreover, memory recall is associated with greater activity in medial regions of the DMN in both, healthy subjects and AD patients (Dhanjal et al. 2014). It is therefore plausible that the excitatory rTMS protocol we applied here could have reinforced this memory-related cortical mechanism by increasing PC activity.

From a neurobiological perspective, rTMS could have induced clinical improvement by promoting changes in synaptic plasticity, the most important biological mechanism for learning and memory. Long-term potentiation (LTP) is considered as a main neurophysiological correlate of these cognitive functions (Bliss and Lømo 1973). We recently demonstrated that AD patients show a disruption in LTP-like cortical plasticity since early clinical stages (Koch et al. 2012; Di Lorenzo et al. 2016). In this context, high-frequency rTMS might have induced LTP-like cortical plasticity within the PC of our cohort of AD patients. Consistent with this hypothesis, TMS-EEG analysis revealed a specific increase of PC neural activity. This enhancement was evident not only at a local but also at a network level. Indeed, changes in neural activity were located over two distinct clusters of electrodes: one corresponding to the site of stimulation (PC), one corresponding to the medial frontal cortex, suggesting that rTMS induced relevant modulations over a medial parieto-frontal circuit. Interestingly, the topography of this EEG network resembles the anatomical distribution of the DMN, as identified by functional MRI (Buckner et al. 2008; Raichle et al. 2001). We also found that rTMS induced an enhancement of TMS-evoked beta activity, both in terms of power and phase synchronization, focused over the medial parietal electrodes underlying the site of rTMS delivery. Since we applied rTMS at 20 Hz, a frequency that falls within the range of beta oscillations, our results could be explained by a possible long-lasting entrainment of beta-rhythm induced by rTMS (Rosanova et al. 2009). In this perspective, our findings are in agreement with models proposing beta activity as an efficient cortical frequency through which the brain networks communicate relevant information, playing a pivotal role on different memory processes (Feurra et al. 2016).

The main limitation of this study is the relatively small sample size. However, in order to select a homogeneous cohort, the clinical diagnosis of AD was supported by the use of CSF biomarkers in all participants, according to the current diagnostic criteria (Dubois et al. 2016). In addition, the low number of electrodes used for EEG recordings limits the spatial resolution of our conclusions, especially for the source analysis. In spite of these limitations, to our knowledge, this is the first study that investigated both the behavioral and neurophysiological effects of an rTMS protocol in AD patients. From a methodological point of view, we demonstrated the reliability of the TMS-EEG approach in revealing specific cortical changes that were not detectable by the resting EEG analysis.

In conclusion, our results show novel evidence that rTMS may be a potential effective strategy for treating patients with early AD for whom, currently, there is no available therapy. Our work is in line with an emerging framework considering circuit-based dysfunctions as a model for cognitive impairment (Canter et al. 2016), and identifies the PC as a novel interventional target to successfully improve memory in AD.

Funding

This work was supported by grant of the Italian Ministry of Health (grant number RF-2010-2311484 to MB and GK).

References

Bäckman L, Small BJ, Fratiglioni L. (2001) Stability of the preclinical episodic memory deficit in Alzheimer's disease. Brain 124: 96-102.

Bentwich J, Dobronevsky E, Aichenbaum S, Shorer R, Peretz R, Khaigrekht M, Marton RG, Rabey JM (2011) Beneficial effect of repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease: a proof of concept study. J. Neural. Transm. 118:463-71.

Bliss TV, Lømo T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J. Physiol. 232: 331-56.

Bonnì S, Veniero D, Mastropasqua C, Ponzo V, Caltagirone C, Bozzali M, Koch G. (2015) TMS evidence for a selective role of the precuneus in source memory retrieval. Behav Brain. Res. 282: 70-5.

Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA. (2005) Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J. Neurosci. 25: 7709-17.

Buckner RL, Andrews-Hanna JR, Schacter DL. (2008) The brain's default network: anatomy, function, and relevance to disease. Ann. N. Y. Acad Sci 1124: 1-38.

Bzdok D, Heeger A, Langner R, Laird AR, Fox PT, Palomero-Gallagher N, Vogt BA, Zilles K, Eickhoff, SB. (2015) Subspecialization in the human posterior medial cortex. Neuroimage 106: 55-71.

Canter RG, Penney J, Tsai LH. (2016) The road to restoring neural circuits for the treatment of Alzheimer's disease. Nature 539: 187-196.

Casula EP, Testa G, Bisiacchi PS, Montagnese S, Caregaro L, Amodio P, Schiff S. (2017a) Transcranial Direct Current Stimulation (tDCS) of the Anterior Prefrontal Cortex (aPFC) Modulates Reinforcement Learning and Decision-Making Under Uncertainty: a Double-Blind Crossover Study. J. Cogn. Enh. 1:318-326.

Casula EP, Stampanoni Bassi M, Pellicciari MC, Ponzo V, Veniero D, Peppe A, Brusa L, Stanzione P, Caltagirone C, Stefani A, Koch G. (2017b) Subthalamic stimulation and levodopa modulate cortical reactivity in Parkinson’s patients. Parkinsonism Relat. Disord. 34: 31-37.

Casula EP, Bertoldo A, Tarantino V, Maiella M, Koch G, Rothwell JC, Toffolo GM, Bisiacchi P. (2017c) TMS-evoked long-lasting artefacts: a new adaptive algorithm for EEG signal correction. Clin. Neurophy. 128: 1563-74.

Chen Y, Liu Z, Zhang J, Chen K, Yao L, Li X, Gong G, Wang J, Zhang Z. (2017) Precuneus degeneration in nondemented elderly individuals with APOE ε4: Evidence from structural and functional MRI analyses. Hum. Brain Mapp. 38: 271-82.

Cotelli M, Manenti R, Cappa SF, Geroldi C, Zanetti O, Rossini PM, Miniussi C. (2006) Effect of transcranial magnetic stimulation on action naming in patients with Alzheimer disease. Arch Neurol. 63:1602-4.

Cotelli M, Manenti R, Cappa SF, Zanetti O, Miniussi C. (2008) Transcranial magnetic stimulation improves naming in Alzheimer disease patients at different stages of cognitive decline. Eur J Neurol. 15:1286-92.

Cotelli M, Fertonani A, Miozzo A, Rosini S, Manenti R, Padovani A, Ansaldo AI, Cappa SF, Miniussi C. (2011) Anomia training and brain stimulation in chronic aphasia. Neuropsychol rehabil. 21:717-41.

Daselaar SM, Prince SE, Dennis NA, Hayes SM, Kim H, Cabeza R. (2009) Posterior midline and ventral parietal activity is associated with retrieval success and encoding failure. Front. Hum. Neurosci. 3: 13.

Delorme A, Makeig S. (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134: 9-21.

Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS. (2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31: 968-80.

Dhanjal NS, Wise RJ. (2014) Frontoparietal cognitive control of verbal memory recall in Alzheimer's disease. Ann. Neurol. 76: 241-51.

Di Lorenzo F, Ponzo V, Bonnì S, Motta C, Negrão Serra PC, Bozzali M, Caltagirone C, Martorana A, Koch G. (2016) Long-term potentiation-like cortical plasticity is disrupted in Alzheimer's disease patients independently from age of onset. Ann. Neurol. 80: 202-10.

Donohue MC, Sperling RA, Salmon DP, Rentz DM, Raman R, Thomas RG, Weiner M, Aisen PS. (2014) The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. JAMA Neurol. 71: 961-70.

Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. (2016) Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement. 12: 292-323.

Ferrucci R, Mameli F, Guidi I, Mrakic-Sposta S, Vergari M, Marceglia S, Cogiamanian F, Barbieri S, Scarpini E, Priori A. (2008) Transcranial direct current stimulation improves recognition memory in Alzheimer disease. Neurology. 71:493-8.

Feurra M, Galli G, Pavone EF, Rossi A, Rossi S. (2016) Frequency-specific insight into short-term memory capacity. J. Neurophysiol. 116: 153-8.

Gili T, Cercignani M, Serra L, Perri R, Giove F, Maraviglia B, Caltagirone C, Bozzali M. (2011) Regional brain atrophy and functional disconnection across Alzheimer's disease evolution. J. Neurol. Neurosurg. Psychiatry 82: 58-66.

Huijbers W, Vannini P, Sperling RA, Cabeza R, Daselaar SM. (2012) Explaining the encoding/retrieval flip: memory-related deactivations and activations in the posteromedial cortex. Neuropsychologia 50: 3764-74.

Klimesch W. (1997) EEG-alpha rhythms and memory processes. International Journal of Psychophysiology 26: 319-40.

Koch G, Di Lorenzo F, Bonnì S, Ponzo V, Caltagirone C, Martorana A. (2012) Impaired LTP- but not LTD-like cortical plasticity in Alzheimer's disease patients. J. Alzheimers Dis 31: 593-9.

Lundstrom BN, Ingvar M, Petersson KM. (2005) The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. Neuroimage 27: 824-34.

Maris E, Oostenveld R. (2007) Nonparametric statistical testing of EEG- and MEG-data. J. Neurosci. Methods 164: 177-90.

Mancini M, Mastropasqua C, Bonnì S, Ponzo V, Cercignani M, Conforto S, Koch G, Bozzali M. (2017) Theta Burst Stimulation of the Precuneus Modulates Resting State Connectivity in the Left Temporal Pole. Brain Topogr. 30:312-319.

Nguyen JP, Suarez A, Kemoun G, Meignier M, Le Saout E, Damier P, Nizard J, Lefaucheur JP. (2017) Repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease. Neurophysiol Clin. 2017 Feb;47(1):47-53.

Nilakantan AS, Bridge DJ, Gagnon EP, VanHaerents SA2 Voss JL. (2017) Stimulation of the Posterior Cortical-Hippocampal Network Enhances Precision of Memory Recollection. Curr. Biol. 27: 465-70.

Pascual-Marqui RD, Esslen M, Kochi K, Lehmann D. (2002) Functional imaging with low-resolution brain electromagnetic tomography (LORETA): a review. Methods Find. Exp. Clin. Pharmacol. 24(Suppl C): 91-95.

Rabey JM, Dobronevsky E, Aichenbaum S, Gonen O, Marton RG, Khaigrekht M. (2013) Repetitive transcranial magnetic stimulation combined with cognitive training is a safe and effective modality for the treatment of Alzheimer's disease: a randomized, double-blind study. J Neural Transm; 120:813-9.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. (2001) A default mode of brain function. Proc. Natl. Acad. Sci. U S A 98: 676-82.

Rastogi A, Cash R, Dunlop K, Vesia M, Kucyi A, Ghahremani A, Downar J, Chen J, Chen R. (2017) Modulation of cognitive cerebello-cerebral functional connectivity by lateral cerebellar continuous theta burst stimulation. NeuroImage. 158:48-57.

Rosanova M, Casali A, Bellina V, Resta F, Mariotti M, Massimini M. (2009) Natural frequencies of human corticothalamic circuits. J. Neurosci. 29: 7679-85.

Rose NS, LaRocque JJ, Riggall AC, Gosseries O, Starret MJ, Meyering EE, Postle BR. (2016) Reactivation of latent working memories with transcranial magnetic stimulation. Science. 354: 1136-9.

Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis F, Di Iorio R, et al. (2015) Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic

principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin. Neurophysiol. 126: 1071-107.

Stokes MG, Chambers CD, Gould IC, Henderson TR, Janko NE, Allen NB, Mattingley JB. (2005) Simple metric for scaling motor threshold based on scalp-cortex distance: application to studies using transcranial magnetic stimulation. J Neurophysiol. 94: 4520-7.

Tadel F, Baillet S, Mosher JC, Pantazis D, Leahy RM. (2011) Brainstorm: a user-friendly application for MEG/EEG analysis. Comput. Intell. Neurosci. 8.

Turriziani P, Smirni D, Zappalà G, Mangano GR, Oliveri M, Cipolotti L. (2012) Enhancing memory performance with rTMS in healthy subjects and individuals with Mild Cognitive Impairment: the role of the right dorsolateral prefrontal cortex. Front Hum Neurosci. 6.

Vannini P, Hedden T, Becker JA, Sullivan C, Putcha D, Rentz D, Johnson KA, Sperling RA. (2012) Age and amyloid-related alterations in default network habituation to stimulus repetition. Neurobiol. Aging 33: 1237-52.

Wagner AD, Shannon BJ, Kahn I, Buckner RL. (2005) Parietal lobe contributions to episodic memory retrieval. Trends Cogn. Sci. 9: 445-53.

Wang JX, Rogers LM, Gross EZ, Ryals AJ, Dokucu E, Hermiller MS, Voss JL. (2014) Targeted enhancementof cortical-hippocampal brain networks and associative memory. Science. 345: 1054-7.

Table 1. Neuropsychological assessment at baseline of AD patients.

Cognitive Domain	**Neuropsychological test**	**score**
LONG TERM MEMORY		
Verbal memory	**Free and cued selective reminding test**	
	Immediate Free Recall (cut-0ff ≥19.59)	17.0 (1.3)
	Immediate Total Recall (cut-off ≥35)	26 (3)
	Delayed Free Recall (cut-off ≥6.31)	4.0 (1.2)
	Delayed Total recall (cut-off ≥11)	8 (1.7)
	Short story test	
	Immediate Recall (cut-off ≥3.1)	3.9 (2.7)
	Delayed recall (cut-off ≥2.6)	3.6 (2.3)
Visuo spatial memory	**Rey's Complex figure**	
	Immediate recall (cut-off ≥6,4)	11.2 (8.1)
	Delayed recall (cut-off ≥6.3)	7.9 (4.7)
SHORT TERM MEMORY		
Verbal	Digit span (≥3.7)	4.8 (1.3)
Spatial	Corsi block tapping task (3.5)	4.1(0.6)
LANGUAGE	Naming of object from BADA (≥22)	28.0(3.5)
REASONING	Raven's Progressive Matrices (≥18.9)	26.2 (6.3)
EXECUTIVE	Modified Card Sorting test	4.4 (0.7)

FUNCTIONS	criteria achived (cut-off≥4.2)	
	Modified Card Sorting test perseverative errors (cut-off≥7.6)	6.0 (2.0)
	Phonological verbal fluency(≥17.3)	33.8 (7.3)
PRAXIS ABILITIES	Copy of drawing (cut-off ≥7.1)	9.5 (1.3)
	Copy of drawing with landmarks cut-off ≥61.8	67.0 (1.8)
ATTENTION	Trail Making Test A (≤94 sec)	76 (13)
	Trail Making Test B (≤283 sec)	136 (21)
	Trail Making Test A-B (≤187 sec)	85 (17)

The table shows the mean (SD) performance scores obtained on neuropsychological testing. For each administered test appropriate adjustments for gender, age and education were applied according to the Italian normative data. Available cut-off scores of normality (≥ 95% of the lower tolerance limit of the normal population distribution) are also reported for each test.

Table 2. Principal Demographic and Clinical Characteristics of AD patients.

AGE, years (mean ±SD)	70.0 ± 5.1
SEX ,% female	50
EDUCATION, years (mean ±SD)	7.2 ± 3.0
MMSE (mean ±SD)	26.1 ± 1.8
ADL (mean ±SD)	5.6 ± 0.5
IADL (mean ±SD)	7.3 ± 0.6
CDR (mean ±SD)	0.3 ± 0.3
Disease duration (months)	13.8 ± 5.1
CSF beta 1-42 pg/mL (mean ±SD)	383.1 ± 16.2
CSF total tau pg/mL(mean ±SD)	558.3 ± 190.7
CSF p-tau pg/mL(mean ±SD)	72.8± 16.2
APOE ε4+ %	65

Abbreviations: AD=AD; MMSE: Mini-Mental State Examination; ADL: Activities of daily Living; IADL: Instrumental activities of Daily living; CSF: cerebrospinal fluid; SD: Standard deviation.

Cognitive test	pre rTMS	post rTMS	pre sham	post sham	ANOVA Group x time
MMSE	26.9± 1.9	27.3± 1.6	25.8± 2.1	26.7± 2.6	*p*=n.s.
RAVLT (IR)	25.4 ±6.5	26.4 ±7.7	25±8.0	25.9 ±8.2	*p*=n.s.
RAVLT (DR)	2.2 ± 2.7	3.0 ± 2.6	2.4 ±2.7	2.4 ±2.9	*p*=0.02*
FAB	14.1 ± 2.2	13.9±1.9	14.0±2.2	14.3±2.5	*p*=n.s.
DSST	89.4± 2.7	90.3± 1.7	89.3± 2.5	89.2± 2.7	*p*=n.s.

Table 3. Cognitive performances of AD patients in different experimental conditions.

Abbreviations: rTMS: repetitive transcranial magnectic stimulation; MMSE: Mini-Mental State Examination; RAVLT: Rey Auditory Verbal learning Test (IR: Immediate Recall; DR: Delayed Recall); DSST: Digit Symbol Substitution Test.

Figure legends

Figure 1. Experimental design and source analysis reconstruction.

(A) AD patients (n=14) were randomly assigned to either a first neuronavigated rTMS or control stimulation (sham) arm. rTMS/sham was daily applied in a 10-session course, Monday to Friday, for a total duration of two weeks. A two-week washout interval was then applied, following which patients were crossed over to the alternative study arm for two additional weeks. The order of assignment to either study arm was counterbalanced across patients. (**B**) rTMS was applied over the PC at 20 Hz (1600 stimuli per day), using a neuronavigation system to ensure that the same spot was constantly stimulated across sessions. (**C**) Source analysis of TMS-evoked EEG activity showed a bilateral activation of the PC, as reconstructed at the peak fit of cortical response between 60 and 90 ms from PC stimulation with single-pulse TMS.

Figure 2. Behavioral results.

(**A**) Learning curves for the 15 word-list of the Rey Auditory Verbal Learning Test before and after two weeks of treatment with rTMS (left panel) or sham (right panel). (**B**) Immediate (IR) and (**C**) Delayed Recall (DR) of the Rey Auditory Verbal Learning Test before and after two weeks of treatment with rTMS or sham. * $p<0.05$. Error bars indicate SEM.

Figure 3. TMS/EEG evoked responses in the spatio/temporal domain.

(A) rTMS, but not sham **(B)** increases cortical activity from 60 to 90 ms (red thick line) following a single-pulse TMS applied over the PC. Such increase is spatially distributed over the electrodes located on the medial fronto-parietal areas, as shown by the scalp maps of surface voltage distribution. (**C, D**) No significant effects are detectable when conducting the same analysis on the cortical response after single-pulse TMS of the l-PPC. *$P<0.05$. Line shading indicated SEM

Figure 4. TMS/EEG evoked responses in the time/frequency domain: ERSP

(**A**) rTMS, but not sham (**B**), enhances PC beta activity in terms of spectral power, as revealed by event-related spectral perturbation (ERSP). No significant effects are detectable when the same analysis is conducted on the l-PPC (**C,D**). *P<0.05. Error bars indicated SEM.

Figure 5. TMS/EEG evoked responses in the time/frequency domain: ITC

(**A**) rTMS, but not sham (**B**), synchronized PC beta oscillatory activity, as revealed by inter-trial coherence (ITC). No significant effects are detectable when the same analysis is conducted on the l-PPC (**C,D**). *P<0.05. Error bars indicated SEM.